ORIGINAL ARTICLE

Rapid recovery of *Mycobacterium tuberculosis* complex from clinical specimens using the BACTEC 9000 MB system, a new automated fluorimetric technique

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Objective: To evaluate the new non-radioactive automated method BACTEC 9000 MB system for the rapid detection of mycobacteria in clinical specimens.

Methods: Ninety clinical specimens from 90 patients with a clinical diagnosis of tuberculosis were tested by both BACTEC 9000 and standard microbiological methods, and the results compared.

Results: The BACTEC 9000, in comparison with the standard method, showed significantly higher detection rates (45 of 90 positive versus 34), shorter time to culture positivity (mean time 18.8 versus 27.4 days) and lower contamination rate (2.2% versus 5.5%).

Conclusions: These results encourage the use of this new system and suggest its use in microbiological laboratories involved in mycobacteriology.

Key words: *Mycobacterium tuberculosis* complex, BACTEC 9000, rapid diagnosis of tuberculosis

INTRODUCTION

In recent years the incidence of tuberculosis has shown, all over the world, a dramatic resurgence, mainly in high-risk populations such as AIDS and other immunocompromised persons, chronic alcoholics, the homeless and drug abusers [1,2]. The diagnosis of tuberculosis due to *Mycobacterium tuberculosis* (MTB) is most commonly made by using both microscopy and culture. Microscopy has low sensitivity and specificity and can provide at best only a preliminary diagnosis. Traditional culture media (Middlebrook agar or Löwenstein–Jensen medium) take 4–8 weeks before a final diagnosis can be made. This approach may result in missed or delayed diagnosis, often allowing spread of infection. In order to improve the clinical management of mycobacterial infections, rapid methods have been developed to shorten the time required for isolation and identification of suspected *Mycobacterium* isolates from clinical specimens [3,4].

The BACTEC 9000 MB system (BACTEC 9000) is a new, non-radioactive method developed for the rapid detection of mycobacteria from clinical specimens. The system uses a traditional broth medium for the growth of mycobacteria and incorporates an oxygen-quenched fluorescent indicator for microbial growth detection. Actively respiring mycobacteria consume the dissolved oxygen and produce a bright fluorescence which is automatically read by the instrument. In this study we compare BACTEC 9000 and conventional culture methods for the recovery rate and the time required for the detection of the MTB complex in clinical specimens, and also the culture contamination rate.

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MATERIALS AND METHODS

Clinical specimens

We examined 90 specimens obtained from 90 patients in the Ospedale Cotugno, Bari, Italy, with a clinical diagnosis of tuberculosis. The clinical specimens processed for isolation of mycobacteria included 56 sputa, one bronchoalveolar lavage fluid, 19 bronchial aspirates, seven pleural fluids, and seven urines.

Processing of clinical specimens

Clinical specimens were processed by standard methods [5]. Briefly, sputum and other respiratory secretions were liquefied with *N*-acetyl-L-cysteine. All contaminated-site specimens were decontaminated for 15 min with NaOH (final concentration, 2%) and centrifuged at 3500*g* for 15 min. Urine was concentrated by centrifugation at 3500*g* for 15 min and the sediment was decontaminated as described above. Normally sterile body fluids (i.e. pleural fluids) were concentrated by centrifugation at 3500*g* for 30 min and cultured without prior decontamination [6]. The sediment obtained from all specimens was suspended in 0.067 M phosphate buffer (pH 6.8) to a final volume of 2.0 mL. Smears were prepared from all the suspensions and thoroughly examined for the presence of acid-fast bacilli (AFB) after Ziehl–Neelsen (ZN) staining.

Media and culturing methods

Aliquots (0.25 mL) of each specimen were used to inoculate Löwenstein–Jensen (LJ) medium. The tubes were incubated at 37 °C and inspected for 8 weeks at shorter time intervals than usual, in order to synchronize these observations with those obtained by BACTEC 9000. In addition, aliquots of 0.5 mL were inoculated into vials of BACTEC 9000 Myco/F (Becton & Dickinson, Cockesville, Md). These vials consist of a fluorescent indicator embedded in silicone on the bottom of bottles filled with 40 mL of an enriched BBL Middlebrook 7H9 broth base with 0.25% glycerol. Each BACTEC Myco/F culture vial was supplemented with 2 mL of an antimicrobial mixture (PANTA) containing polymyxin B (400 000 U/L), amphotericin B (70 mg/L), nalidixic acid (280 mg/L), trimethoprim (70 mg/L), and azlocillin (80 mg/L). The vials are flushed with 10% CO_2, capped with polypropylene caps and sterilized by autoclaving. In line with the manufacturer's guidelines, the vials were incubated at 37 °C and read automatically, every 10 min, on the BACTEC 9000 instrument for 42 days. Growth was detected by measuring the fluorescence present in the vial; this is directly proportional to the amount of oxygen consumed by bacterial growth. When the vials became positive for fluorescence production, acid-fast staining was performed. If AFB were seen in the smears, the BACTEC 9000 vial was considered positive for mycobacteria and subcultured on LJ medium to allow further identification by the niacin test. In addition, a plate of trypticase soy agar with 5% sheep blood was inoculated in order to exclude culture contamination.

RESULTS

Forty-five (50%) mycobacterial isolates from 90 clinical specimens tested were detected by either the BACTEC 9000 system or LJ medium. In particular, in the BACTEC 9000 method 36 sputa, one bronchoalveolar lavage fluid, five bronchial aspirates and three urines were positive, whereas in the conventional culturing method 29 sputa, one bronchoalveolar lavage fluid, two bronchial aspirates and two urines gave positive results. Thirty-four isolates were detected by both systems. All the isolates were identified as MTB complex by the niacin test. Of these isolates 41/45 (91.2%) were obtained from ZN smear-positive clinical specimens, while the remaining 4/45 isolates (8.8%) were from ZN smear-negative specimens (i.e. two sputa and two bronchial aspirates). Thirty-four of 90 specimens were positive by conventional methods, and these plus another 11 (45/90) by BACTEC 9000. The contamination rates observed were 2.2% for BACTEC 9000 and 5.5% for LJ medium (data not shown).

When the mean time to detection was calculated, we observed that it was significantly shorter with BACTEC 9000 than with LJ medium (10.8 versus 27.4 days). Furthermore, bacterial isolation by BACTEC 9000 occurred very early when compared with the isolation on LJ medium. Thirty-three strains were detected within the range 1–21 days and one strain on day 38. In contrast, visible growth on solid LJ medium was observed from day 20; 30 strains were detected between 20 and 28 days and four strains were detected within the range 50–60 days (Figure 1). It is noteworthy that the BACTEC 9000 was able to detect MTB complex from smear-negative specimens. Indeed, the four strains of MTB complex derived from smear negative clinical samples were isolated only by BACTEC 9000, within the range 15–36 days.

DISCUSSION

The increase in tuberculosis has prompted the development of more rapid and efficient methods for the isolation of mycobacteria from clinical specimens. The conventional methods currently in use for the cultivation of these microorganisms on solid media require

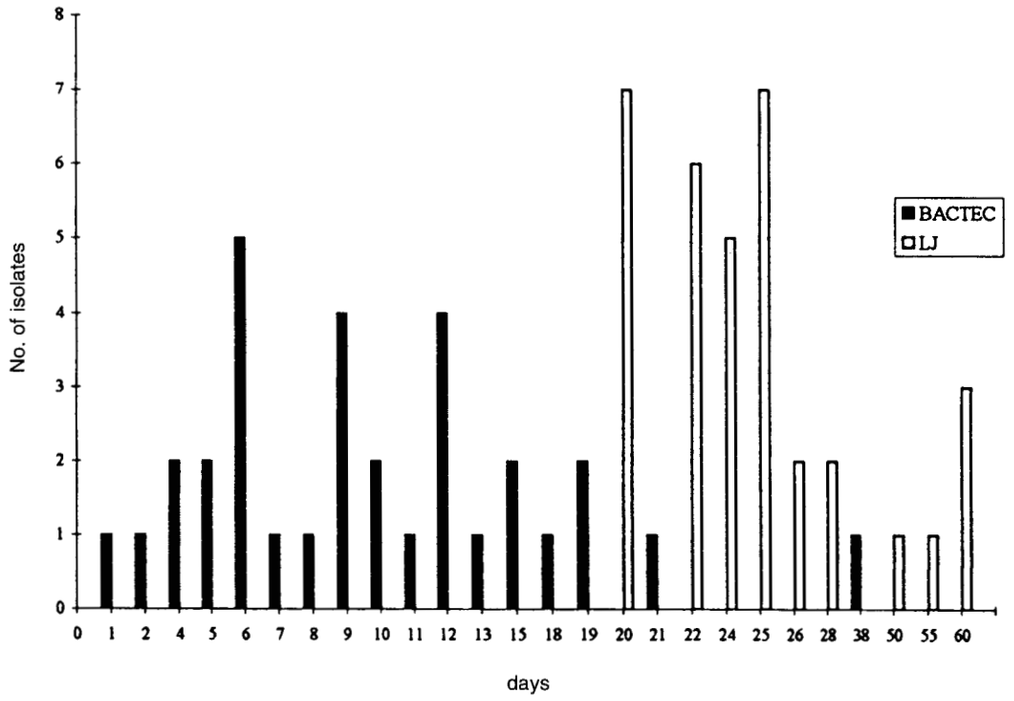

Figure 1 Time (days) required for the isolation of *Mycobacterium tuberculosis* complex: BACTEC 9000 system (BACTEC) versus growing on Lowenstein–Jensen (LJ) medium.

several weeks of incubation and, in addition, they may lack sensitivity when used alone. BACTEC 9000 is a new system which, in addition to a traditional broth medium, uses a novel fluorescent indicator for the recovery of mycobacteria directly from the patient's specimen. In this study, we compared BACTEC 9000 with two conventional methods for direct detection, acid-fast-stained smears and culture on LJ medium. Since one of the goals in mycobacteriology is the development of new methods that expedite the diagnosis, the value of any new method can first be estimated on the basis of the time required for the microorganism's isolation. In this regard, our results have shown that the mean time of mycobacterial detection is significantly shorter when using BACTEC 9000 instead of conventional LJ medium and that nearly all of the positive results were made available within the first 3 weeks of investigation. In addition, BACTEC 9000 also made possible isolation of mycobacteria from four clinical specimens which gave negative results in both ZN microscopy and conventional culture on LJ medium. Although the acid-fast-stained smear is considered the test against which new tests must be measured, the detection limit of microscopy (10^4 bacilli/mL of sputum) is less than that of culture (10^2 bacilli/mL of sputum) [6]. Our data, as far as BACTEC 9000 and LJ are concerned, are consistent with those recently reported by Van Griethuysen et al [7].

The capacity of BACTEC 9000 to detect bacteria which are not detectable using the two indicated methods therefore appears very interesting. The explanation of this result might be the enhanced recovery of mycobacteria in liquid media, as suggested by previous reports showing that liquid media (radiometric BACTEC and Septi-Chek) are significantly superior to conventional solid media for the isolation of MTB and *Mycobacterium avium* complex (MAC) organisms from clinical specimens [8–10]. In our study, LJ slants exhibited a higher rate of contamination than the BACTEC 9000 system. This finding is consistent with recently reported data [11] and confirms that liquid media reduce the growth of contaminating bacteria which eventually escape the decontamination. From the point of view of the laboratory service, BACTEC 9000 does not require disposal of radioactive waste as is the case with BACTEC 460 TB, and therefore the method appears extremely suitable for microbiological laboratories actively involved in mycobacteriology.

References

1. Barnes PF, Bloch AB, Davidson PT, Snider DE Jr. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med 1991; 324: 1644–50.
2. Snider DE Jr, Raviglione M, Kochi A. Global burden of tuberculosis. In Bloom BR, ed. Tuberculosis: pathogenesis, protection, and control. Washington, DC: ASM Press, 1994: 3–11.
3. Sewell DL, Rashad AL, Rourke WJ Jr, Poor SL, MacCarthy JAC, Pfaller MA. Comparison of the Septi-chek AFB and BACTEC systems and conventional culture for recovery of mycobacteria. J Clin Microbiol 1993; 31: 2689–91.
4. Anargyros P, Astill DSJ, Lim ISL. Comparison of improved BACTEC and Löwenstein–Jensen media for culture of mycobacteria from clinical specimens. J Clin Microbiol 1990; 28:1288–91.
5. Nolte FS, Metchock B. *Mycobacterium*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology, 6th edn. Washington, DC: ASM Press, 1995: 400–37.
6. Sommers HM, McClatchy JK, Monella JA. Laboratory diagnosis of the mycobacterioses. Cumitech 16. Washington, DC: ASM Press, 1983.
7. Van Griethuysen AJ, Jansz AR, Buiting AGM. Comparison of fluorescent BACTEC 9000 MB system, Septi-Chek AFB system, and Lowenstein–Jensen medium for detection of mycobacteria. J Clin Microbiol 1996; 34: 2391–4.
8. Pfaller MA. Application of new technology to the detection, identification, and antimicrobial susceptibility testing of mycobacteria. Am J Clin Pathol 1994; 101: 329–37.
9. D'Amato RF, Isenberg HD, Hochstein L, Mastellone AJ, Alperstein P. Evaluation of the Roche Septi-Chek AFB system for the recovery of mycobacteria. J Clin Microbiol 1991; 29: 2906–8.
10. Whittier PS, Westfall K, Sutterquist S, Hopfer RL. Evaluation of the Septi-Chek AFB system in the recovery of mycobacteria. Eur J Clin Microbiol Infect Dis 1992; 11: 915–18.
11. Badak FZ, Kiska DL, Setterquist S, Hartley C, O'Connell MA, Hopfer RL. Comparison of mycobacteria growth indicator tube with BACTEC 460 for detection and recovery of mycobacteria from clinical specimens. J Clin Microbiol 1996; 34: 2236–9.